CARDIOVASCULAR

#910

Comm.

Dr. Bing
Dr. Gardner
Dr. Jacobson

Re: Your Case #154

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

MAY 11 1973

Application for Research Grant
(Use extra pages as needed)

Date: 4 May 1973

1. Principal Investigator (give title and degrees):
James M. Ramsey, Associate Professor of Biology
B.S. degree, Wilmington College (Zoology)
M.S. degree, Miami University (Physiology)
Additional graduate study, University of Cincinnati (Environmental Toxicology)

2. Institution & address:
University of Dayton
300 College Park Avenue
Dayton, Ohio 45469

3. Department(s) where research will be done or collaboration provided:
Biology Department
University of Dayton

4. Short title of study:
The Effects of Chronic Exposure to Low Level Carbon Monoxide on the Red Cell Mass and Blood Viscosity in Human Subjects

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5. Proposed starting date: 1 July 1973

6. Estimated time to complete: One Year

7. Brief description of specific research aims:

Tobacco smoking habits subject individuals daily to intermittent, low level carbon monoxide exposure. Can the slight hypoxemia characteristic of small percentages of persistent carboxyhemoglobin tend to induce acclimation by altering the hematological capacity for oxygen transport?

The major objective of this proposed study is to evaluate whether or not long-term, daily experimental exposures to concentrations of carbon monoxide realistic with those confronting smokers will result in significant polycythemia and increases in blood viscosity in selected human subjects.

Lesser objectives include (a) some elucidation of potential mechanisms of hematological response, e.g., changes in plasma volume and erythropoietic activity; (b) whether or not rates of red cell production and hemoglobin synthesis appear to be closely correlated with each other in response to the stress; and (c) determining the course of hematological changes with time, including whether there is reversibility of incurred changes after exposures have ceased.

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8. Brief statement of working hypothesis:

There is a statistical relationship between long-term smoking habit and cardiovascular disease, and carbon monoxide is one of the tobacco combustion products upon which speculation has centered as a potential factor in this relationship. The role of erythrocytic polycythemia as a potential adaptation to persistent hypoxia may not be an entirely advantageous adaptate. An increased erythrocytic concentration increases the blood viscosity, thereby increasing blood flow resistance. In addition to imposing additional work loads on the myocardium and possibly enhancing risks of thrombus formation, the increased flow resistance can be critical in view of the fact that an increased flow rate is the chief counter-effect in oxygenation of the myocardium when hypoxemia exists (in hypoxemic states other organs increase O_2 extraction, whereas the heart doesn't). In chronic hypoxic hypoxemia (hypobaric O_2) there is a tendency for the red cell mass to increase (polycythemia), and it is thought that a reduced arterial PO_2 triggers erythropoiesis. There is discrepancy in respect to human studies with carbon monoxide hypoxemia,and the relation between intermittent, chronic, low level CO exposures and hematological effects needs resolution. One major difference between the two types of hypoxemia is that with CO the arterial PO_2 is relatively normal, only the O_2 content is affected.

Many studies have indicated that animal exposure to decompression or altitude has resulted in reticulocytosis, erythrocytosis, and elevated hematocrit. Certain studies (Cavusoglu and Kayserilioglu) have shown that such exposures have not appreciably affected hemoglobin synthesis, which suggests that Hb synthesis and cell divisions may be controlled by different mechanisms. Often associated with the increased red cell mass is a plasma volume decrease (Reissmann). Furthermore, animals polycythemic from hypobaric hypoxia show a suppression of RBC production after returning to ambient pressures (Shadduck et al.).

Numerous studies of man at altitude or with decompression chambers likewise have shown a polycythemic response; the immediate effect believed due to a hemoconcentration and continued exposure resulting in elevation of red matter through erythropoiesis. Hurtado has shown that people living at altitude have greater blood viscosity and some macrocytosis of the RBC's. Merino, on the other hand, studying subjects at 4,000 meters, indicated a tendency of erythrocytic microcytosis and a lack of parallelism between increases in RBC and Hb, the former increasing more so. In addition, he found polycythemia to reverse itself when subjects were brought to sea level.

Sanchez studied human subjects at 4,000 meters and found significant elevations in hematocrit and reductions in plasma volume although the total blood volume increased. The polycythemia resembled that seen in patients with congenital heart disease. Billings et al., studied human subjects at 3,800 meters for 20 days. The mean hematocrit increased for two weeks, then stabilized for the remainder of the time.

In exposing animals to CO, some studies have shown elevation of Hb and hematocrit (Jones et al.). This was a continuous exposure (200 ppm for 90 days). Wilks et al., exposed seven dogs daily (six to eight hours) to 80 ppm CO and obtained increases in Hb content and hematocrit. Ramsey found increases in Hb and reticulocyte percentages in rats briefly exposed to high levels of CO (1200 ppm). However, other studies of intermittent and continuous exposures (50 ppm) with rodents and dogs have produced no hematological changes (Lindberg) (Stupfel et al.).

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In respect to hematological effects of CO exposures with man, the picture is even more discrepant. In substantial and prolonged exposures to one human subject, Killick obtained no changes in RBC count, reticulocyte proportion, or in blood volume. Using a limited number of rather high (12 to 30% COHb) intermittent exposures, Ramsey showed some elevation in mean hematocrit and Hb content of eight subjects. However, the elevations were not statistically significant in every exposure. Siggard-Andersen et al., used eight days of exposures (five times per day) resulting in 11% COHb and obtained no significant change in plasma volumes. Kjeldsen and Damgaard exposed eight subjects intermittently for eight to ten days (13% COHb). They obtained a moderate increase in reticulocytes but nonsignificant changes in hematocrit. The same subjects exposed later to 3,500 meters altitude showed a gradual increase in hematocrit. Finne claims that hypoxia, anemic or hypoxic, will result in increased production of erythropoietin within 12 hours. Some writers (Dinman) (Beard) have stated that long-term CO exposures may produce increased hematocrits and Hb, but that available data are inadequate to draw conclusions. Finally, Eisen and Hammond, working with habitual smokers who were asked to refrain from smoking for various periods of time, claim that hematocrits, RBC counts, and Hb were found to be higher during periods of smoking than during abstinence.

It is obvious that it simply isn't clear whether or not chronic, low level, intermittent exposures of CO can result in significant polycythemia or significant increases in blood viscosity. The situation with CO is not nearly so clear as is the case with hypobaric O_2. In anemic hypoxia (which is what CO exposures amount to), the arterial PO_2 is not significantly reduced. If indeed the arterial PO_2 is the primary trigger in erythropoiesis, then perhaps low level CO may not be capable of producing polycythemia.

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9. Details of experimental design and procedures (append extra pages as necessary)

Ten young, male human subjects of the same age would be recruited from a selective screening process. The subjects must be healthy and nonsmokers. Included in the screening process would be examinations for pulmonary performance, EKG, and standard hematology. Permission from each selectee's personal physician is also obtained. The subjects are paid for their services. I have used human subjects in several past studies and am aware of necessary precautions. The University of Dayton has a board for evaluating all research proposals involving human subjects. The subjects in the proposed study will likely be University students. An effort will be made to select subjects whose hematology (RBC count, Hb, hematocrit) shows a stable day by day level, and it is also quite desirable that there is uniformity (slight standard deviation) within the group in respect to hematological values. Rather than use two groups of subjects for exposed subjects and controls, respectively, I shall employ just one group and let them be their own controls. In this way there is a perfect match or comparison of exposed versus unexposed effects. By using the ten subjects first as controls, this design is quite feasible and therefore most desirable. Throughout the duration of the study the subjects must adhere to a reasonable dietary and drinking regime, and must possess daily and weekend habits that prevent any prolonged or frequent sojourns in automobile traffic.

The first seven weeks of the study would be spent in recruiting, screening, and selecting subjects as well as obtaining adequate supplies and performing preliminary operations.

Beginning with the eighth week, data recording would begin. The ten subjects would be subjected twice daily (45 minutes each time) to normal air in our large, walk-in, environmental chamber. During the time in the chamber, the subjects may read, play cards, or otherwise leisurely pass the time. They are never told at any time about the composition of gaseous material they breathe. Exposure to normal air twice daily each weekday would continue for three months. Every third day, 2 ml of antecubital vein blood is withdrawn from each of two subjects immediately upon leaving the second session in the chamber. Each time it is a different two subjects until it cycles around. This blood is examined for carbon monoxide by the method of Trinder and Harper, excellent for low ranges. Also, every fourth day, plasma volume determinations are performed on each of two subjects. This is done with antecubital vein blood employing the Evans dye method. This is performed on the same two subjects each time and since 10 ml of blood is required, these two subjects are not used for other hematological evaluations. Also, because of this volume of blood and potential dye retention for two to three days, the determination is done every fourth day. It is performed in the morning (fasting). Also, for five days each week, a battery of hematological evaluations are performed on each of the other eight subjects. These are done at the same time each day (morning with fasting conditions) and are determined from microquantities of capillary blood from finger prick. Less than 0.4 ml of blood is required for the entire series and since microquantities are involved, most of the determinations are done in triplicate. Determinations for Hb content are done with the cyanmethemoglobin method and read spectrophotometrically. Hematocrits are determined with the International Micro-Capillary Centrifuge, Model MB, and the accessory microcapillary reader, Model CR. Erythrocyte counts are determined with a Coulter

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Counter. Reticulocyte percentages are obtained from thin smears with new methylene blue staining (Color Index 927-Wintrobe). Percentages from 15 different slide locations are averaged. Blood viscosity is determined by means of the viscosimeter of Hess, based on Poiseuille's law. The test requires only a drop of blood and can be performed in 30 seconds.

After three months of exposure to normal air (controls) and daily determinations of the hematological series in the eight subjects, all subjects are then subjected twice a day to the chamber in the very same manner except that now the chamber has a concentration of 300 ppm carbon monoxide in air. Everything else is identical to the first three months subjection to the chamber. The subjects do not know what they're breathing and the technicians do not know anything about the exposures (hematological determinations are performed at a different time and in a different room). Therefore, a double psychological blind exists throughout the study. 300 ppm CO for two periods of 45 minutes each should provide an accumulation of 5 to 8% carboxyhemoglobin each day (reversed each night). This is realistic with that found in heavy smokers by the end of a day. The chamber is loaded from a tank of 100% CO with two-stage regulator and precise flow meter setting to adjust the chamber interior to a desired concentration of CO. The chamber is exhaustable and can be cleared. I have had considerable experience with such exposure techniques with human subjects. Air in the chamber can be monitored (unknown to occupants) for CO concentration. The twice daily CO exposures are done each weekday for six months. Blood samples are taken for CO content and plasma volume determinations (same two subjects as during the first three months) as was described for the control runs. Also, the five days a week hematological series (Hb, hematocrit, RBC count, reticulocyte percentage, and blood viscosity) is determined each morning. After the six months of CO exposures, the hematological series and the plasma volume determinations are continued for an additional month.

The Biology Department has computer facilities so that data reduction and statistical analyses can be performed readily. Most of the tests applicable to the type data of the proposed study are parametric. Even though close attention will be given individually to the ten subjects, the results of the study must be viewed with considerable statistical inference. Eight to ten subjects should be sufficient in view of the fact that they are serving as their own controls, which reduces variance considerably. Therefore, much attention will be given to comparing means for each treatment and the testing for significance of mean differences with paired "t" values in before-after treatment on the same subject. Also, much of the data would lend itself to correlation-regression application; e.g., degree of hematological change with time, or correlating degree of change in one hematological parameter with that of another. Nonparametric (chi-square) procedures may be employed to evaluate whether or not subjects show change, irrespective of degree.

With no unforeseen difficulties, the data may certainly be worth publishing, perhaps in the Journal of Applied Physiology or "Blood".

Depending on the data obtained this first year, additional study may be in order to further elucidate points of the first year's data.

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Some of the points worth considering in subsequent years would be the role of erythrocytic 2,3, diphosphoglycerate concentrations in response to carbon monoxide exposures as well as evaluating more specific involvement of erythropoietin and its mechanisms in regulating the red cell production, Hb synthesis, etc., in carbon monoxide exposures. Erythropoietic suppression, RBC destruction, and Hb catabolic rates in the liver would offer still other related avenues of investigation.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

Two modern, air conditioned research laboratory areas in the Biology Deparment are under the direction of the proposed Principal Investigator for studies in respiratory physiology, hematology, and environmental physiology. The main laboratory, Sherman 125, is equipped with instrumentation for blood gas analyses, pulmonary performance testing, cardiac and circulatory evaluations, and standard hematology. This laboratory also has extensive files of reprints related to CO toxicology.

The second laboratory area, Sherman 229, is where exposure chambers for both humans and animals are housed. The human environmental chamber is a huge, walk-in facility comfortably capable of handling ten individuals at a time. Temperature and relative humidity within are precisely controlled. For the most part, very little in the way of additional equipment is needed for the proposed study. An additional hemophotometer for Hb evaluations could expedite processing eight daily determinations, i.e., two technicians could share the eight determinations with two instruments. Also, an additional centrifuge (handling 15 ml tubes, $120) could be used in working with plasma volume determinations.

11. Additional facilities required:

None

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12. Biographical sketches of investigator(s) and other professional personnel (append):

See pages 8 and 9.

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

See pages 12 and 13.

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12. Biographical Sketch

JAMES M. RAMSEY — Principal Investigator

Title: Associate Professor of Biology, University of Dayton - Environmental Physiologist

Age: REDACTED

Education: B.S. degree, Wilmington College (Zoology), 1948;
M.S. degree, Miami University (Physiology), 1951;
Additional graduate study, University of Cincinnati (Environmental Toxicology), 1957-1959.
Because of my research reputation, everyone presumes I have terminal degree; which I don't.

Experience: Associate Professor, Department of Biology, University of Dayton, 1970;
Assistant Professor, Department of Biology, University of Dayton, 1964;
Medical Research Associate, University of Cincinnati College of Medicine, 2 years;
Instructor and Research Associate, Physiology, Miami University, Oxford, Ohio, 5 years;
Instructor, Biology, Cedarville College, Cedarville, Ohio, 4 years

Professional Membership Affiliations:

REDACTED REDACTED

Listed Recognition: American Men of Science; Dictionary of International Biography

Past Research Grants: National Science Foundation, Research Related to Carbon Monoxide Toxicology, July 1966-1967, $1,600;
National Science Foundation, Research Related to Carbon Monoxide Toxicology, July 1967-1968, $2,000;
Public Health Service (NAPCA), Research Related to Carbon Monoxide Toxicology, July 1968-Nov. 1969, $11,037;

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Public Health Service (NAPCA), Research Related to Carbon Monoxide Toxicology, December 1969-April 1971, $12,682;
National Science Foundation, Research Related to Carbon Monoxide Toxicology, July 1971-1972, $3,000;
National Science Foundation, Research Related to Carbon Monoxide Toxicology, November 1972-1973, $2,825

Publications: The above studies have resulted in 10 publications (one in press), seven of which are cited below.

"Carboxyhemoglobinemia in Parking Garage Employees," J. M. Ramsey, Arch. Environ. Health, Vol. 15, November 1967.

"Potassium Pallado Sulfite Detection of Carbon Monoxide in Exhaled Air as an Estimate of Carboxyhemoglobin," J. M. Ramsey, Amer. Indust. Hyg. Assoc. J., Vol. 28, December 1967.

*"The Immediate Haematological Response in the Rat to Experimental Exposures of Carbon Monoxide," J. M. Ramsey, Jour. Physiol., 202:297-304, 1969.

*"The Time Course of Hematological Response to Experimental Exposures of Carbon Monoxide," J. M. Ramsey, Arch. Environ. Health, 18:323-329, March 1969.

*"Oxygen Reduction and Reaction Time in Hypoxic and Normal Drivers," J. M. Ramsey, Arch. Environ. Health, 20:597-601, May 1970.

*"Carbon Monoxide, Tissue Hypoxia, and Sensory, Psychomotor Response in Hypoxaemic Subjects," J. M. Ramsey, Clinical Science, 42:619-625, May 1972.

"The Effects of Single Exposures of Carbon Monoxide on Sensory and Psychomotor Response," J. M. Ramsey, Amer. Indust. Hyg. Assoc. J., 1973 (in press).

*Reprint included with proposal.

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14. First year budget:

A. *Salaries (give names or state "to be recruited")*	% time	Amount
Professional (give % time of investigator(s) even if no salary requested)		
J. M. Ramsey	100% - 2 summer months 10% Academic Year	REDACTED
Technical		
Thomas M. Fitzsimmons	20% all year	REDACTED
Gregory MacNealy Part-Time Lab Technicians		
Staff Benefits		
Sub-Total for A		REDACTED

B. Consumable supplies (by major categories)

2 Tanks, 100% Carbon Monoxide (Matheson), $7.50 ea.	$ 15
20 Vials Pre-Cal Hematocrit Tubes, $2.50 ea.	50
200 Disposable Syringes and Needles	25
20 Vials, 20 Lambda Disposable Blood Pipets, $13 ea.	260
Chemical Reagents	50
Sub-Total for B	$ 400

C. Other expenses (itemize)

Remuneration for 10 Experimental Subjects, $100 ea.	
Sub-Total for C	$ 1,000
Running Total of A + B + C	REDACTED

D. Permanent equipment (itemize)

S-51720 Centrifuge (Sargent)	$ 120
2-675-150V1 Hemophotometer (Fisher)	300
Sub-Total for D	$ 420
E	$ 1,362
Total request	REDACTED

E. Indirect costs (15% of A+B+C)

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	REDACTED	$422	$1,200	0	$1,458	REDACTED
Year 3		$445	$1,200	0	$1,529	

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16. Other sources of financial support.

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
The Effect of Chronic, Low Level Carbon Monoxide Exposure on the Erythron of the Rat. (Graduate student thesis as part of study.)	University of Dayton	$2,825	November 1972-1973

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
	NONE		

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made.

Principal investigator

Typed Name James M. Ramsey

Signature J M Ramsey Date Apr. 9, 1973

Telephone (513) 229-3011
Area Code Number Extension

Checks payable to

Controller, University of Dayton

Mailing address for checks

300 College Park Avenue

Dayton, Ohio 45469

Responsible officer of institution

Typed Name Joseph W. Stander

Title Authorized Representative

Signature [signature] Date 4/13/73

Telephone (513) 229-2246
Area Code Number Extension

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BIBLIOGRAPHY

Altland, P. D. and B. Highman. "Acclimatization Response of Rates to Discontinuous Exposures to Simulated High Altitudes," Am. J. Physiol., 167:261, 1951.

Beard, R. R. "Toxicological Appraisal of Carbon Monoxide," J. Air Poll. Cont. Assoc., 19(9):722, September 1969.

Billings, C. E., et al. "Medical Observations During 20 Days at 3,800 Meters," Arch. Environ. Health, 18:987, June 1969.

Cavusoglu, H. and A. Kayserilioglu. "The Effects of General Hypoxia and Renal Ischemia on Erythropoietin Production," Arch. Intern. de Physiol. et de Biochim., 77:260, 1969.

Dinman, B. D. "Effects of Long-Term Exposure to Carbon Monoxide," Nat. Acad. of Sciences, Washington, D. C., 1969, p. 25.

Eisen, M. E. and E. C. Hammond. "The Effect of Smoking on Packed Cell Volume, Red Blood Cell Counts, Haemoglobin, and Platelet Counts," Canad. Med. Assoc. J., 75:520, September 1956.

Finne, P. H. "Hematologic Compensation Mechanisms in Hypoxia," Norsk Laegeforening, Tidsskrift 91:194, 1971.

Fried, W., Johnson, C., and P. Heller. "Observations on Regulation of Erythropoiesis During Prolonged Periods of Hypoxia," Blood, 36(5):607, November 1970.

Hurtado, A., Merino, C., and E. Delgado. "Influence of Anoxemia on the Hemopoietic Activity," Arch. Intern. Med., 75:284, May 1945.

Jones, R. A., et al. "Effects on Experimental Animals of Long-Term Inhalation Exposure to Carbon Monoxide," Toxicol. and Appl. Pharmacol., 19:46, 1971.

Killick, E. M. "The Nature of the Acclimatization Occurring During Repeated Exposure of the Human Subject to Atmospheres Containing Low Concentrations of Carbon Monoxide," J. Physiol., 107:27, 1948.

Kjeldsen, K. and F. Damgaard. "Influence of Prolonged Carbon Monoxide Exposure and High Altitude on the Composition of Blood and Urine in Man," Scand. J. Clin. and Lab. Invest., 22:20, 1968.

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Lait, S., McDonald, T. P., and R. D. Lange. "Effect of Hypoxia on Body Weight, Body Water, and on Hematological Values in Mice," Lab. Animal Care, 20(3):483, 1970.

Merino, C. F. "Studies on Blood Formation and Destruction in the Polycythemia of High Altitude," Blood, V(1):1, January 1950.

Ramsey, J. M. "The Immediate Haematological Response in the Rat to Experimental Exposures of Carbon Monoxide," J. Physiol., 202:297, 1969.

Ramsey, J. M. "The Time Course of Hematological Response to Experimental Exposures of Carbon Monoxide," Arch. Environ. Health, 18:323, March 1969.

Reissmann, K. R. "Blood Volume in the Dog During Altitude Acclimatization," Amer. J. Physiol., 167:52, October 1951.

Reissmann, K. R., Burkhardt, W. L., and B. Hoelscher. "Blood Destruction in the Polycythemia Induced by Hypoxia," Blood, 7(3):337, 1952.

Sanchez, C., Merino, C., and M. Figallo. "Simultaneous Measurement of Plasma Volume and Cell Mass in Polycythemia of High Altitude," J. Appl. Physiol., 28(6):775, 1970.

Shadduck, R. K., et al. "Regulation of Erythropoiesis (XXIV). Studies on the Post-Hypoxic Rebound Phase," Blood, 34(4):477, October 1969.

Siggaard-Andersen, J., Petersen, F. B., and T. I. Hansen. "Vascular Permeability and Plasma Volume Changes During Hypoxia and Carbon Monoxide Exposure," Angiology (Scand.), 20:356, June 1969.

Stupfel, M. and G. Bouley. "Physiological and Biochemical Effects on Rats and Mice Exposed to Small Concentrations of Carbon Monoxide for Long Periods," Ann. New York Acad. Sci., 174:342, October 1970.

Trinder, P. and F. E. Harper. "A Colorimetric Method for the Determination of Carboxyhaemoglobin Over a Wide Range of Concentrations," J. Clin. Path., 15:82, 1962.

Wilks, S. S., Tomashefski, J. F., and R. T. Clark, Jr. "Physiological Effects of Chronic Exposure to Carbon Monoxide," J. Appl. Physiol., 14(3):305, 1959.

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